

Stearic acid solubility and cubic phase volume

Walter F. Schmidt*, Justin R. Barone, Barry Francis, James B. Reeves III

Animal and Natural Resources Institute, Beltsville Agricultural Research Center, ARS, USDA, Beltsville, MD 20705, United States

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Abstract

Stearic acid (SA) is highly soluble in structurally diverse solvents. SA/solvent packing within a $(24.8 \text{ \AA})^3$ cubic volume explains the stoichiometry of SA solubility at multiple temperatures in multiple solvents. In the absence of solvent, the cubic volume contains 25 molecules at van der Waals distances from each other. At 55°C , SA occupied half the cubic volume in saturated solution of four structurally diverse solvents. Below 4% SA/volume (e.g. in acetonitrile), the head and foot of each SA molecules on average is more than one solvent molecule away from the head and foot of a neighboring SA molecule. At 50% SA/cubic volume, $-\text{CH}_2-$ groups on SA molecules are separated from neighboring $-\text{CH}_2-$ groups on SA molecules by a monolayer of solvent molecules. Lowering the temperature from 55 to 25°C , the volume fraction of SA decreased by a factor of 2 (or more) for every 6°C . Lowering temperature increased the relative number of column of solvent molecules in the cubic phase, and correspondingly, the distance between SA molecules within the cubic volume increased. In three of five solvents, molecular mechanics calculations demonstrated the van der Waals stabilization that occurs from SA/SA affinity in the absence of solvent is similar in magnitude to the van der Waals stabilization from SA/solvent affinity. Methyl-*t*-butyl ether was less stabilized than hexane, acetone or methanol because the more bulky molecules packed less efficiently within the cubic volume. The most efficient/most stable packing however was still as columns of solvent between columns of SA. The efficiency and stability of SA and solvent packing optimal within the $(24.8 \text{ \AA})^3$ cubic volume. Between 100 and 8% SA, multiple SA molecules present within the cubic volume function as SA aggregates. Both inter- and intra-cubic (phase) volume properties of SA aggregates coexist.

Although acetonitrile and SA at the molecular level are both rod shaped, acetonitrile disrupted the packing of SA molecules within the cubic phase. The disrupted packing explains the much lower solubility of SA in acetonitrile than in the other solvents. The same molecular structures (e.g. methanol) can either stabilize or disrupt the packing of aggregated SA molecules, depending upon temperature. The mechanisms of aggregation within cubic volumes could also occur with structurally more complicated lipids. Aggregation and dispersion from such cubic phases could also be present in more complex chemical and/or macromolecular environments.

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1. Introduction

Lipids and biochemical environments can interact with a high degree of specificity in the presence of hydrophilic and/or hydrophobic chemical structures. Docosahexaenoic (DHA) acid is a polyunsaturated lipid essential in the development of human brain structure

* Corresponding author at: 10300 Baltimore Avenue, Bldg. 012 Rm. 1-5, Beltsville, MD, United States. Tel.: +1 301 504 6765; fax: +1 301 504 5992.

E-mail address: schmidt@ba.ars.usda.gov (W.F. Schmidt).

and function, but docosapentaenoic acid (DPA), the lipid missing one double bond from DHA, is not part of brain structure (Crawford et al., 1999). The stability and biological activity of macromolecular proteins can be changed in the presence of a structurally different lipid (Gouaux and White, 2001). Crystallization of membrane protein from solution can require addition of structurally specific lipids (Caffrey, 2003). The molecular structure and conformation of the natural polyunsaturated lipid can alter the closeness in space of neighboring structurally different lipids (Broadhurst et al., 2004). Thus a molecular and structural basis may exist by which lipids stabilize or alter the conformation of adjacent biochemical molecules.

Molecular level interactions have been shown to occur among structurally different lipids in the presence of a hydrophilic aqueous phase. Monounsaturated acylglycerol/water (Qui and Caffrey, 1999) and monostearylglycerol/water (Chupin et al., 2001) each can form gel, liquid crystal and cubic phases. The molecular size and weight of the saturated fatty acid and the length of lipid polar head groups altered the relative amounts of the lamellar and the non-lamellar phase which form (Lewis and McElhaney, 2000). The relative concentration among cationic, anionic and zwitterion lipids affected the lipid surface charge which in turn changed the amount of non-lamellar phase present (Caboi et al., 2001). Additional hydrophobic or hydrophilic compounds added to a monounsaturated fat/water mixture induced a marked phase changes within the bilayer including in the lipid phase composition (Yue et al., 2003). Changing the lipid/water ratio or the lipid composition alters the physical properties of the phases that form. Therefore, this lipid phase depends upon the molecular structure of the lipid(s) present and the properties of the water present. Lipids however also associate in the absence of water.

Changes in the molecular structure among the lipids affect the observed macroscopic physical properties. Changes in the lipid composition membranes affected the permeability of long-chain fatty acids transport into and out of phosphatidylcholine vesicles (Thomas et al., 2002). Synthetic homogeneous triacylglycerols were less soluble in phosphatidylcholine vesicles than natural heterogeneous triacylglycerols (Li et al., 2003). The fatty acids within triacylglycerols can be structurally redundant. The potential of intra-molecular phase transitions and intra-molecular structural redundancy complicate interpretation of lipid interactions.

Stearic acid (SA), a long C₁₈ straight-chain saturated fatty acid, has been found to bind and plasticize

“green” composites (Netravali, 2003), human serum albumin (Bhattacharya et al., 2000) and α -helical sites in biomolecules (Vila et al., 1998). Association, however, can also occur with structurally less complicated ligands. Common organic solvents that readily solubilize SA differ in chemical structure and hydrophobicity. Methanol, acetonitrile, acetone, methyl-*t*-butyl ether (MtBE), and hexane were selected as ligands. The ligand/substrate ratios at five different temperatures and molecular mechanics were used to investigate the mechanism(s) and structural basis for hydrophobic and hydrophilic binding of substrates to SA ligands.

2. Experimental

2.1. Methods: GLC

Stearic acid was added to approximately 10 ml of each of the selected solvents in screw cap test tubes that were then covered with Teflon tape and capped. The test tubes were placed in a covered water bath so that the solution was below the water level and held the selected temperature. The solutions were intermittently stirred on a vortex mixer. After 48 h an aliquot was taken from each solvent, placed in a weighed glass scintillation vial, immediately capped, and weighed. The aliquots were stored at 3 °C while awaiting analysis. After allowing the aliquots to come to room temperature, a known weight of lauric acid, corresponding to the expected amount of stearic acid in the aliquot, was added to each vial and they were diluted to with dichloromethane so that the lauric acid concentration would be approximately 0.45 nmoles/ μ l. The diluted aliquots were then analyzed by gas chromatography.

Gas chromatographic analysis was carried out on a Hewlett Packard 5890 Series II gas chromatograph equipped with a flame ionization detector and a Model 7673 autoinjector (Hewlett Packard, Palo Alto, CA). The injector and detector temperatures were 300 °C. A Nukol (bonded poly(ethylene glycol) modified with nitroterephthalic acid) fused silica column (15 m \times 0.53 mm i.d., 0.50 μ m film thickness) (Supelco, Inc., Bellefonte, PA) was programmed from 110 °C (2 min) to 200 °C at 10 °C/min. Helium was used as the carrier gas at an initial flow rate of 1 ml/min. Six 1 μ l splitless injections were made for each solution with a solvent blank between each sample run.

A series of injections of known concentration of lauric and stearic acid were made on the GC to develop a standard curve for concentration versus peak area for each of the compounds. The equations from the standard curve were used in conjunction with the lauric acid

addition for determination of the stearic acid concentration of the aliquots. Solubility was measured in 6 °C intervals for each of the five solvents from 25 to 55 °C.

2.2. Methods: molecular mechanics

The stable lowest energy 3D conformation of an aggregate of 25 molecules of SA was generated in HyperChem 5.11 (Hypercube, Gainsville, FL) using MM+ force fields (Zhdanov et al., 2003). Geometric optimization was then used to find the local minimum on the potential energy surface for more than one molecule. The conjugate gradient (Polak-Ribieri) method was used as the optimizer, and RMS gradient of 0.01 was used for considering the optimization to be converged. Multiple experiments from different initial orientation among molecules were performed and the relative orientational changes among the molecules that consistently increased or decreased stabilization energy were identified. The lowest energy conformation among molecules was used as a lead conformation from which smaller systematic variations in the orientations were investigated. The potential energy surface was taken as a global minimum when any perturbation in the conformation or orientations of SA or solvent increased surface energy.

The frame of reference was an empty volume in which SA completely fits in any X, Y and Z direction. MM+ determined its unit dimension as a cubical box with a unit dimension of 24.8 Å. For each solvent (hexane, MtBE, acetone, acetonitrile and methanol), MM+ was used to determine the number of solvent molecules that occupied the same length as one SA molecule. The distances between solvent molecules in multiple orientations were optimized by MM+ and the number of solvent molecules that equals 24.8 Å column length was determined. The central SA molecule in the MM+ optimized cubic volume was deleted and replaced by a column of solvent molecules. MM+ calculations then optimized the van der Waals distance between solvent molecules within the hydrophobic SA chemical environment. The optimization was repeated from multiple starting solvent molecule conformations until no additional improvement in packing among the solvent molecules surrounded by SA was detected.

The number of solvent columns (replacing SA molecules) was increased within the volume until the experimentally determined stoichiometric ratio solvent to SA was achieved. Columns of solvents were dispersed among SA molecules to preclude an optimized structure based upon non-uniform mixing of solvent and solute. The same molecular force field parameters were used in the SA/solvent cubic volumes as in the SA cubic volume.

3. Results and discussion

3.1. Cubic shape and cubic phase volume of SA

The shape of the unit volume required to adequately describe the packing of SA and solvents is a cube. At the molecular level, the smallest volume occupied by a SA molecule is a flattened rod. The maximum length of each SA is 24.8 Å and the resulting rod has a volume of about 49.9 Å³. Thus 25 molecules of SA in a 5 × 5 array at van der Waals distances equals the volume of a cube. It is the maximum number of SA molecules that can fit within a cubic volume that has the unit dimension of one SA molecule: (24.8 Å)³ = 15,253 Å³. Twenty-five SA molecules optimized within a cubic volume using molecular mechanic calculations (Fig. 1A1 (top view) and A2 (side view)) demonstrate the most efficient packing among the molecules. Correspondingly, the optimum stabilization due to van der Waals attractive forces occurs at distances in which the molecules are most efficiently packed.

The colligative properties from the self-association of lipids into a cubic phase alter its physical–chemical properties relative to that of the individual molecule. Translational diffusion of individual lipid molecules within a cubic phase or between two cubic phase volumes is much slower than the diffusion of individual molecules

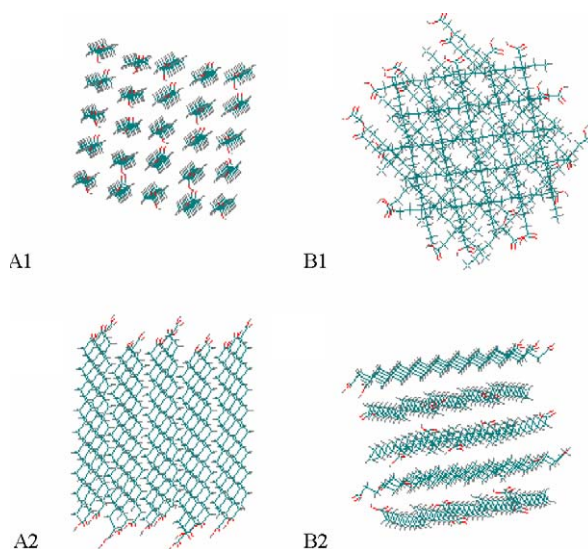


Fig. 1. Top and side views of two arrays of SA molecules contained in cubic phase volumes. (A1) top view with the rows aligned and parallel to the columns. (A2) Side view of A1 showing columns are also aligned and parallel. (B1) Top view with rows are perpendicular in adjacent columns. (B2) Side view of B1 showing columns remain aligned and parallel. Conformation labeled A is lower energy than conformation labeled B from its more efficient packing.

in the absence of the aggregate phase; quenching of fluorescence dyes dissolved in the lipid phase is slower in the aggregate phase than in the absence of the phase (Lindblom et al., 1992). Viscosity is related to molar volume (partial molar volume in binary mixtures) (Richet and Polian, 1998). In aggregates, viscosity is related to n molar volumes where $n > 1$.

The stabilization in the 25 molecules SA cubic volume was -341 kcal per cubic volume (-13.6 kcal/mole SA molecule), primarily due to van der Waals forces between $-\text{CH}_2-$ groups on SA molecules adjacent to each other. The molecular forces are not exactly equal among all 25 molecules. The 4 corner SA molecules have only 2 adjacent SA molecules; the 16 SA molecules along the outside of the cube are adjacent to 3 SA molecules; the central 9 SA molecules each are adjacent to 4 SA molecules.

Both staggering among the ends of aligned SA molecules and bending among aligned SA molecules decreased the stabilization of the aggregate. Both decreased the number of van der Waals contacts between molecules. SA columns on average fit uniformly aligned and parallel into the same cubic phase volume (Fig. 1A2). From this beginning three types of conformational changes could perturb the cubic phase volume: (1) one or more columns could bend or tilt widening individual SA columns; (2) one or more SA molecules within a column could drift upward or downward within a column; (3) a SA molecule could twist along the short axis within the column. The average unit volume of the cube increased in cases (1) and (2) because correspondingly packing was less efficient. In case (3) only a minor change in properties of the cube occurred because rotation within a cylinder does not change the volume of the cylinder.

Each increase in localized pockets of non-uniformity created void volumes that in turn also increased the volume of the unit cube. Every time dependent incident of non-uniformity within the cubic volume occurs with equal probability at any 1 of the 25 SA molecules. Any local inhomogeneity at one site must be averaged with a similar local inhomogeneity at each of the 25 SA molecules within the unit cell. The net effect of 25 local perturbations within the cubic volume would be a small increase the average van der Waals distance among all of the 25 SA molecules. The cubic volume would remain a cubic volume.

Each SA molecule has a hydrophobic $-\text{CH}_3$ head and a polar $-\text{COOH}$ foot resulting in a molecular dipole moment. Molecular mechanics calculations confirmed head/foot–foot/head pairing stabilized the molecular forces within the cubic volume. The net dipole moment

for the cube is essentially the same as for only one molecule of SA. On average for every 13 head groups of SA on 1 surface of the cube, 12 will be on the opposite cube. Head/foot–foot/head associations minimize head/head interaction among SA molecules both within the cubic volume and between adjacent cubic volumes.

In contrast if the head groups of all 25 SA molecules were on the same surface of a cube, the 25 dipole moments would be parallel. The surface properties of one face of the cube would be stabilized by hydrogen bonding forces between $-\text{OH}$ and $\text{C}=\text{O}$ groups but $-\text{CH}_2-$ packing among the long side chains would be less efficient. Dipolar asymmetry in one cubic volume aligned with a second similar cubic volume would result in an aggregate containing up to 50 molecules of SA.

The most stable conformation in the cube is when all 25 molecules align in both rows and columns. Within the same cube, any row or any column of five SA molecules can rotate. Rotation of any column or row by 180° decreased the energy of stabilization (anti-parallel dipolar moments become parallel). Rotation another 180° restored the energy of stabilization. Rotation of one or more rows of SA by 90° or -90° had no appreciable effect on the stabilization energy from long-chain SA molecules aligning.

Each column (or each row) can be rotated 45° from the previous row (or column) and the energy of stabilization calculated (Fig. 1B). This higher energy state is due to fewer van der Waals contacts among $-\text{CH}_2-$ groups between columns (or rows), i.e. to -303 kcal per the 25 SA molecules. The new shape of the volume from the rotation was a cylinder. The volume occupied by 25 SA molecules increased because the new cylinder is larger than the initial cube. Thus in part the inter-molecular forces over the volume were weaker because the molecules are on average further apart.

A lipid cubic phase has been identified from crystallographic data: the unit cell contains one whole SA molecule across opposite corners of a cube and multiple truncated fractions of SA molecules filling in the remainder of the volume (Richet and Polian, 1998). The truncated units are at either 45° or 90° angles from the single SA molecule. No $-\text{COOH}$ or $-\text{CH}_3$ groups from any other SA molecule however occur in the unit cell.

The identical structural components are contained within the structure generated from the cubic volume (Fig. 1B). Assigning the SA molecule in the middle of the cylinder as the intact single SA molecule within the

cubic phase, the remaining unit cell can be constructed. The relative position of the remaining truncated $-\text{CH}_2-$ groups is precisely the same as the crystallographic data reported. No other $-\text{COOH}$ or $-\text{CH}_3$ groups would show up in any lipid crystallographic unit cell because every one of the remaining 24 SA molecules would have their $-\text{COOH}$ or $-\text{CH}_3$ groups inside the cylinder, but outside of the crystallographic unit cell.

A structural assignment of the actual position of the $-\text{COOH}$ and $-\text{CH}_3$ groups from the other 24 SA molecule inside the cylinder cannot be predicted. Since the relative angle of rotation between any two rows (or columns) is totally random, the end of the SA molecule in a cubic or cylindrical volume will always be unpredictable: $-\text{COOH}$ is as likely as $-\text{CH}_3$ to occur at any specific location. Crystallographic analysis requires chemical structures present at specific sites to be detectable. In contrast, the $-\text{CH}_2-$ backbone is in the same structural location independent of the location of the end groups.

3.2. Difficulties with spherical model for SA volume fraction

Unlike cubes, spheres cannot pack uniformly without large spaces of undefined void volumes. Mathematically a sphere has close to half the volume of the cube whose length is the diameter of the circle. This is a critically important factor because saturated solutions of SA in this study routinely contained up to half the volume as SA. With seven molecules of solvent equal in volume to one molecule of SA, the solvent molecules all could fit in the sphere, and all could fit within the void volume, but the SA molecule could only fit into the sphere. Another solution is that some solvent molecules would be in the void volume and others in the sphere. With a larger number of solvent and SA molecule, the problem becomes even more complicated.

Only one SA molecule can exist unbent within a spherical volume, i.e. across its diameter of a SA molecule. Adding a second, a third or a fourth SA molecule to the same spherical volume (e.g. by rais-

ing the temperature at which the solution is saturated) becomes increasingly and irrationally more difficult. Solvents could solvate the more highly bent SA molecules more effectively than the less bent molecules, or not. Solvents like hexane could partially straighten bend SA molecules, or not. Void volume could decrease with increasing SA concentration, or not. Solvents effects could decrease the energy requirement to fit SA molecules into the spherical volume, or not. The unavoidable reason the spherical model is untenable is that 12 molecules of SA cannot simultaneously fit into a sphere with a diameter of 24.8 Å, but easily fit into the same diameter cubic volume.

3.3. Stoichiometry and saturated solutions of SA

The maximum temperature for all solvents was selected as the boiling point of MtBE. The melting point of SA (69–70 °C) is in the same temperature range as the boiling points of the solvents: hexane 69 °C, acetonitrile 82 °C, MtBE 55 °C, acetone 57 °C, and methanol 65 °C (Saludjian and Reiss-Husson, 1980). The temperature range in this study was from 55 to 25 °C in 6 °C steps. Reproducibility of the ratio of SA/solvent among all temperatures was less than 2%. The solubility data at 25 °C was more variable than at 55 °C probably because thermal control is more difficult near ambient temperatures.

For all the solvents except acetonitrile, the number of solvent molecules in the saturated solutions was within the same order of magnitude as the number of SA molecules (Table 1). At 55 °C, 2.3 molecules of hexane, 1.7 of MtBE, 4.1 of acetone, and 7.5 of methanol were present for each molecule of SA. In contrast, 213 molecules of acetonitrile were present per each molecule of SA.

Each 6 °C increase in temperature lowered the solvent/SA ratio in saturated solutions by about half or more than half. An explanation for the larger number of acetonitrile molecules at 55 °C compared to the other solvents is that it has a significantly higher boiling point.

Table 1
Mole ratios of solvent to stearic acid in saturated solutions at six temperatures

Solvent	55 °C	49.5 °C	43 °C	36 °C	31 °C	25 °C
Hexane	2.30 ± 0.02	5.16 ± 0.12	11.6 ± 0.1	30.4 ± 0.3	76.4 ± 0.6	208 ± 1
Acetonitrile	213 ± 1	419 ± 2	804 ± 14	1665 ± 9	2180 ± 10	6730 ± 160
MtBE ^a	1.65 ± 0.04	2.93 ± 0.04	4.61 ± 0.04	10.4 ± 0.2	12.6 ± 0.1	28.8 ± 0.3
Acetone	4.11 ± 0.09	8.57 ± 0.05	23.7 ± 0.3	47.4 ± 0.4	91.0 ± 0.8	180 ± 0.8
Methanol	7.48 ± 0.10	13.0 ± 0.2	28.3 ± 0.1	119.6 ± 0.8	207 ± 0.7	592 ± 19

^a MtBE = Methyl-*t*-butyl ether.

Table 2
Stabilization of SA in saturated solutions with structurally diverse solvents

Solvent	Calc'd <i>N</i> molecules solvent per cubic phase volume	Exp'd <i>N</i> ratio solvents per SA molecule (<i>T</i> = 55 °C)	Exp'd <i>N</i> molecules solvents per 25 SA volume	Exp'd <i>N</i> moles solvent in mixed cubic phase	Exp'd <i>N</i> columns SA in mixed cubic phase	Solvent van der Waals stabilization (kcal/mole cubic phase volume)	Stabilization relative to solvent-free SA cubic phase ^a (%)
Hexane	75	2.30 ± 0.02	58	29	13	–297	89.2
Acetonitrile	150	213 ± 1	^b	^b	^b	0.0	0.0
MtBE	100	1.65 ± 0.04	41	28	18	–113	33.1
Acetone	125	4.11 ± 0.09	103	52	13	–376	110.1
Methanol	175	7.48 ± 0.10	187	90	12	–320.	93.8

^a van der Waals stabilization for 25 SA molecules in a cubic phase: –341 kcal.

^b Two hundred and thirteen acetonitrile molecules already exceed the cubic phase volumes of 25 columns of SA.

Assuming that rate is similar for temperatures above 55 °C, the 213 solvent molecules per SA at 55 °C would be reduced to 13 molecules per SA at 79 °C and 6.5 molecules per SA at 85 °C.

The solubility of SA in a solvent above its boiling point was not attempted. Solvent flash points are safety hazard. In addition this study was designed to measure solubility of SA in a liquid phase, not in a pressurized vapor phase. The solubility of SA in acetonitrile at the higher temperatures was not measured in this study because this would be the only sample in which the solvent would be above the melting point of SA.

Nucleation occurs both in the saturated solution phase and in the pure liquid phase. The nucleation of SA occurs because the attractive forces between two or more properly aligned SA molecules are close enough for aggregation to occur. Solvents are dispersive forces that cause localized closeness in space to be temporary and reversible. The temperature of saturated solutions is lower than the SA melting point. This suggests that the solvents may lower the temperature at which SA becomes a solid by solvating and dispersing SA molecules.

3.4. Cubic phase in saturated solutions at 55 °C

A maximum average number of solvent molecules in one dimension will be as long as one SA molecule. From MM+ calculations, the number of solvent molecules that equal the length of one SA molecule can be calculated (Table 2 and Fig. 2). This number ranged from three for hexane to seven for methanol. MM+ optimized

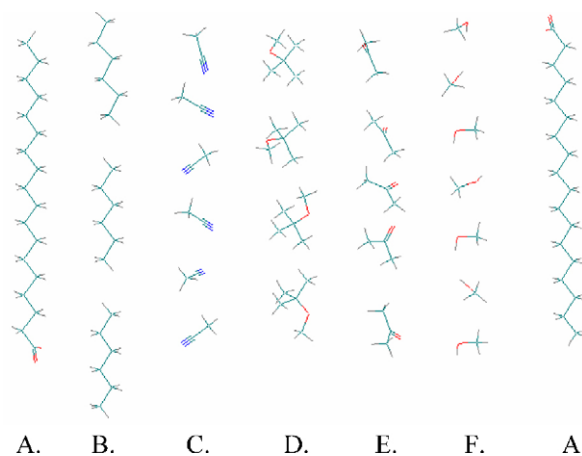


Fig. 2. Same scale: solvent molecules per SA length. (A) Length view of SA. (B) Three hexane molecules. (C) Six acetonitrile molecules. (D) Four methyl *t*-butyl ether molecules. (E) Five acetone molecules. (F) Seven methanol molecules. Adjacent solvent molecules in the column are van der Waals distances from each other.

the distance and most stable conformation of solvent molecules. MM+ calculations demonstrated molecular forces did not favor acetonitrile molecules remaining aligned in a column. In contrast, methanol remained stable in a column by hydrogen bonding pair-wise to one neighboring methanol molecule.

The number of molecules per column times 25 equaled the number of solvent molecules that could fit into the cubic phase volume as columns. The volume of solvent molecules in this space would be equal to the cubic phase volume occupied by 25 SA molecules. The experimental stoichiometry of saturated solutions then determined the ratio of SA present, i.e. in the same volume. At 55 °C, the volume fraction of the cube for each of the individual solvents (except acetonitrile) was half solvent, half SA.

Stoichiometry determined the relative number of SA and solvent molecules in a unit cell, but not the distribution of SA and solvent molecules within the cell. For example, at the number ratio acetone/SA of 4.11, within a unit volume of 15,253 Å³, 52 molecules of acetone (12 columns) and 13 molecules of SA fit. Each column had at least four acetone molecules and four columns had five acetone molecules.

One possibility is that the solvent molecules self-associate and that SA molecules self-associate. Existing as two separate pockets of localized concentration (Fig. 3A1 and A2), three immediate problems from postulating localized populations of solvent arose. First, if localized pockets of solvents are present, there is no reason to assume any stoichiometry exists. Pockets could just as well be a little larger or smaller. Secondly, boundary conditions that would result in reproducible distinct pockets of solvent (or of SA) would require non-uniform molecular forces or a non-uniform distribution of molecular forces to explain this non-uniformity. Thirdly, because each cubic phase volume in solution is adjacent to six other cubic phase volumes, any increased closeness of SA pockets between two cells would correspondingly cause a decrease in closeness to SA in the other adjacent cells. For example, in the conformation in Fig. 3A, an adjacent cell could result in 25 SA molecules being close in space and/or 25 columns of acetone being close in space. Strong solvent/solvent affinity and strong SA/SA affinity would be a mechanism to explain such localized pockets of solvent and/or SA. For a stoichiometry of saturated solution to exist, a ratio of volumes of SA to volumes of solvent must be a constant.

Alternatively, the volume over which solvent and SA are uniform can be contained within the same unit volume, i.e. a cubic volume with the unit dimensions of one SA molecule. Thirteen SA molecules fully solvated by

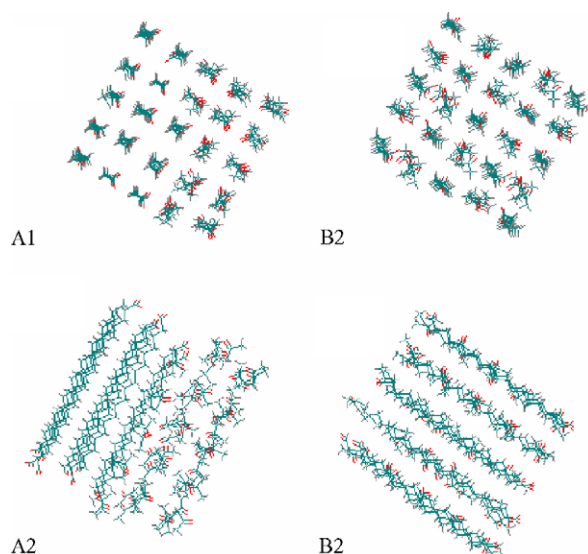


Fig. 3. Top and side views of two arrays of SA molecules in acetone (saturated solution at 55 °C). (A1) Top view of cubic phase containing both SA and acetone localized region within the space. (A2) Side view of A1 showing localized solvent and SA regions. (B1) Top view of same stoichiometry with solvent fully dispersed among SA molecules. (B2) Side view of B1 showing columns acetone fully dispersed among SA molecules. The van der Waals attractive forces in conformation B (solvent + SA) are of similar magnitude to the same forces in the same volume containing only SA (Fig. 1A1 and A2).

12 column of acetone molecules is the maximum entropy and maximum dispersion of SA within the cubic volume (Fig. 3B). The stabilization energy from replacing half the SA molecules with acetone molecules (−376 kcal per unit volume) was similar to that of the volume with only SA present. Thus molecular forces solvating SA are similar in magnitude to the van der Waals forces stabilizing SA in the same cubic volume.

Structural differences among of acetone, methanol and hexane failed to explain the similarity in SA solubility in each of the solvents. In contrast computational chemistry results demonstrated that MtBE also fitted the same model of monolayer SA solvation, but with a much lower energy of stabilization (−81.7 kcal versus −341 kcal for only SA). The cross-sectional area of a column of MtBE however was larger than a similar cross-sectional area of SA. Thus the volume fraction of MtBE was larger than the SA fraction. Also, the shape and bulkiness of MtBE in restricted volumes decreases the capacity to pack efficiently. The less efficient the packing, the fewer the number of van der Waals site that will simultaneously be in contact with each other.

For a saturated solution of SA in acetonitrile, the same cubic volume could not contain 213 acetonitrile molecules even without any SA molecules. Computa-

tional chemistry demonstrated weak affinity between acetonitrile and SA. Indeed, molecules of acetonitrile placed within the cubic volume in place of single SA molecules readily disrupted the cubic array. Solvent/solvent interactions appear to disfavor alignment of molecules (perpendicular or parallel) with SA or with each other. Despite being rod shaped at the molecular level, acetonitrile lacks affinity for the rod shaped SA molecule (and for other acetonitrile molecules).

A change in SA solubility among solvents due to a change in solute chemical potential is responsible for changes in the mole fraction solubility of two component mixtures with temperature at constant pressure (Windholz and Sudavari, 1983). In these cases however, volume (not pressure) was the parameter that was constant. The results suggested that when the partial volume of solvent and SA are equal, the chemical structure of the solvent has little or no influence on the properties of the SA. Any difference between solvents would be related to the temperature at which SA and the solvent have an equal partial volume. With acetonitrile, for example, the temperature at which SA occupies half the volume of a saturated solution would need to be above 79 °C instead of at 55 °C. At a critical temperature (and pressure), a specific number of solvent molecules in a saturated solution can occupy precisely the same volume as SA.

3.5. Saturated solutions at lower temperatures

Lowering the temperature decreased the number ratio of solvent to SA in the saturated solutions. Each 6 °C decrease lowered the solvent/SA ratio by about half. Within a volume containing 25 SA molecules, half the volume would equal about 12 SA molecules. Half again reduces that to six SA molecules, half of which is then three molecules of SA. The lowest concentration of SA possible within the same cubic volume is one molecule of SA. In the cubic volume, the total volume is a constant and only the ratio of the solvent and SA vary as one changes temperature. Within the unit cubic volume, one SA molecule equals 4% of the total volume. With 50% of the cubic volume SA, solvents and SA ideally contribute equally to the properties of the saturated solution.

On decreasing temperature, more solvent molecules are required to maintain a saturated solution. Solution properties include increasingly more solvent/solvent interactions. The molecular forces that enable 200 molecules of methanol and 200 molecules of acetonitrile to solvate a single molecule of SA are most certainly similar. This mole ratio however occurs in methanol at 31 °C, but at 55 °C for acetonitrile. A comparison of the molecular forces (per molecule) between methanol or

acetonitrile and SA at the molecular level is valid only the stoichiometry is known. For example, SA within acetonitrile at 55 °C and SA within methanol at 31 °C have a fully unexpected similarity in their stoichiometry.

Molecular dynamics (kT) are a function of temperature where k is Boltzman's constant. Yet methanol at 25 °C perturbs SA association within a cubic phase, but at 55 °C the same molecular structure favors SA self-association. There are 79 times fewer molecules of methanol in the cubic volume at 55 °C than at 25 °C, even if each methanol molecule moves predictably faster 55 °C than at 25 °C. Thus molecular interactions as a function of temperature or free energy of mixing with temperature can be intrinsically inaccurate unless changes in stoichiometry with temperature are properly included. The variables dn_{MeOH}/dT and $dn_{\text{MeOH}}/dn_{\text{SA}}/dT$ (where n_{MeOH} and n_{SA} are the moles of methanol and SA, respectively) can be very different from zero even over a small temperature change.

3.6. Homogeneity in solution phase structure of SA

SA molecules are much larger than solvent molecules which are much larger than void volumes. SA solubility is not solvents fitting into a random array of single SA molecules. Nor is it SA molecules fitting into a random array of solvent void volumes. For SA and solvent to be at a specific mole ratio, a volume with molecular dimensions must exist in which the stoichiometry is valid. In saturated solutions containing a random array of single SA molecules, the size and shape of void volumes would be innately inconsistent. The accessibility of solvent molecules would depend upon the degree of inefficiency of packing among SA molecules.

Assuming SA had to fit into spacing among solvent molecules, the larger the number of SA molecules, the more complicated the process of finding space for an additional SA molecule. A large minimum number of solvent molecules must be present to be able to fit additional SA molecule. Since otherwise adding SA to solvent will increase the total volume of the solution, this model could consistently underestimate SA solubility.

The smallest space that can explain the acetone packing from 55 °C than at 25 °C is the $(24.8 \text{ \AA})^3$ cubic volume. In the saturated solutions examined (except in acetonitrile), each cubic volume has the same chemical composition as the stoichiometry. The cubic volume is also the minimum space $(15,253 \text{ \AA}^3)$ in which multiple SA molecules can occur uniformly unbent in solution. Diluting the cubic volume and/or lowering the temperature, the cubic volume remains identical and only the ratio SA/solvent changes. The average distance among

SA molecules increases within the cubic volume until 4% SA. At below 4% SA, less than one SA molecule exists per cubic unit volume and a spherical molecular frame of reference is most appropriate.

SA is much larger in size than solvent molecules. Thus, accounting for SA distribution within a cubic volume must occur before space for solvents or void space can be allocated. In cases in which volume fraction of solvent is much greater than the volume fraction of SA, solvent/solvent effects can be more important than SA/SA effects. The same van der Waals forces that explain SA/SA affinity in the cubic phase of SA (liquid/melt) can also explain SA/solvent affinity in saturated solutions in which SA is more than 8% SA. The cubic phase volume present in liquid SA may be similar to the cubic volume present in solutions containing half solvent/half SA.

In micelles below the critical micelle concentration, every unit cell volume is identical in the chemical composition to its neighboring cell. In aqueous solutions of SA, the unit volume of SA is much larger than in non-aqueous solvents because its solubility in water is much lower. At above the critical micelle concentration, the SA is present at concentration greater than in the aqueous saturated solution. The solution can be polydisperse because the SA concentration in two adjacent unit volumes is not necessarily identical (Castellan, 1983). Homogeneity is monodisperse.

4. Conclusions

Despite the structural differences, hexane, acetone, MtBE, and methanol, solvents occupied 50% of the volume in saturated solutions of SA (55 °C). Molecular mechanics demonstrates that 25 SA molecules fit at van der Waals distances from each other within a $(24.8 \text{ \AA})^3$ cubic volume. On average, a column of solvent molecules uniformly separates parallel SA molecules from each other. The same van der Waals forces that enable SA molecules to associate in a cubic volume enable solvents to associate between the SA molecules.

Acetonitrile is a linear molecule in which SA is two orders of magnitude lower in solubility. Molecular mechanics found van der Waals forces do not stabilize acetonitrile in columns parallel to SA. Indeed, acetonitrile perturbed the packing of SA at molecular distances much larger than a solvent monolayer. Solvents occupy over 96% of the molecular volume (55 °C). In this case, the terminal end of each SA molecule is more than one molecule of acetonitrile away from the terminal end of another SA molecule.

Every about 6 °C lower in temperature (from 55 °C), the relative number of solvent molecules present in saturated solutions of SA doubles. The molecular properties of each molecule of SA are that of individual molecules only at concentrations lower than 4% of the unit cubic volume. At a volume fraction above 4% SA, SA molecules exist as aggregates within each cubic volume. Molecular properties of multiple SA molecules within the cube are separate from the molecular properties of SA molecule aggregates contained within the cubic volume. The cubic volume of SA in saturated solutions (55 °C) corresponds structurally to the cubic phase volume present in SA the absence of solvent.

Macromolecules contain structural features common to those present in solvent molecules. A macromolecule can associate with either a single SA molecules or with the surface of a cubic volume containing an aggregate of SA molecules. Acetonitrile and methanol (25 °C) disrupt SA aggregation in saturated solutions. Similar mechanism of aggregation and dispersion could prove applicable in structurally more complex lipids within structurally more complex chemical environments.

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